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Postharvest Biology and Technology 34 (2004) 11–20
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Integrating heat treatment, biocontrol and sodium bicarbonate to reduce postharvest decay of apple caused by *Colletotrichum acutatum* and *Penicillium expansum*

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Received 25 September 2003; accepted 10 May 2004

Abstract

'Golden Delicious' apples were wound inoculated with conidial suspensions of either *Colletotrichum acutatum* or *Penicillium expansum*, then treated with heat (38 °C) for 4 days, sodium bicarbonate, and/or one of two heat tolerant biocontrol agents (yeasts). Following four months storage at 0 °C, the apples were left at room temperature for two weeks. Populations of antagonists were stable throughout the experiment and were higher on the heated than the non-heated fruit. Both antagonists reduced decay caused by *P. expansum*, whereas heat or heat in combination with either antagonist eliminated decay. Either heat or the antagonists alone reduced decay caused by *C. acutatum*, but a combination of the two was required to completely eliminate decay caused by this pathogen. Adding sodium bicarbonate to the heated or antagonist-treated fruit had little effect on decay caused by either pathogen but when used on non-heated fruit, it significantly reduced decay severity caused by *P. expansum* after four months at 0 °C. The goal of this research is to combine alternative methods of control to provide an effective substitute for synthetic pesticides.
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Keywords: Bitter rot; Blue mold; Biological control; *Metchnikowia pulcherrima*

1. Introduction

Elucidating non-chemical control methods to reduce postharvest decay is becoming increasingly important. Consumers are demanding less chemical residue on produce, and many fungi are developing resistance to commonly used fungicides. The use of fungicides is becoming more restricted due to health concerns (Ragsdale and Sisler, 1994). It is therefore necessary to develop alternatives to synthetic chemical control to reduce environmental risks and raise consumer confidence. Several alternatives show

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0925-5214/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.postharvbio.2004.05.011

promise, but none alone is as effective as fungicides. A strategy must be developed that combines several of these alternatives to enhance their effectiveness.

Prestorage heat treatment is one alternative of increasing interest, for use in alleviating physiological maladies of various commodities (Lurie, 1998). It has shown potential as a method to reduce postharvest decay as well. Heat treatment (38 °C for 4 days) was effective in eradicating *Penicillium expansum* Link. on apples initially but exhibited no residual activity (Fallik et al., 1995). Similarly, 'Golden Delicious' apples inoculated with *P. expansum* and then exposed to 38 °C for 4 days had either significantly less decay than non-heated fruit or the decay was totally eradicated (Leverentz et al., 2000). The same heat treatment also reduced decay caused by *Colletotrichum acutatum* but did not control it as effectively as *P. expansum* (Janisiewicz et al., 2003).

Another alternative that continues to show promise is that of biological control of postharvest diseases (BCPD) (Janisiewicz et al., 2001; Janisiewicz and Jeffers, 1997; Korsten et al., 1994; Usall et al., 2001; Wilson and Wisniewski, 1989; Zhou et al., 2001). Decay caused by *Botrytis cinerea* and *P. expansum* has been controlled on apples and pears by bacterial and yeast antagonists, both under laboratory conditions and in large-scale tests (Chand-Goyal and Spotts, 1996; Janisiewicz et al., 1994; Janisiewicz and Marchi, 1992; Roberts, 1990). Effective biocontrol has also been achieved against postharvest pathogens of stone, citrus, and subtropical and tropical fruit worldwide (Janisiewicz and Korsten, 2002). Several biocontrol agents are now being used commercially to control postharvest decay of fruit. For example, Biosave (EcoScience Corp., Orlando, FL) has been used on pome fruit and Aspire (Ecogen Inc., Langhorne, PA) is being used on citrus fruit. Biosave products, which utilize strains of *Pseudomonas syringae*, have proven successful on pome and citrus fruit; their use has expanded to cherries and potatoes and they are currently being considered for use on other fruit and vegetables, as well.

Another alternative is sodium bicarbonate (SBC, $NaHCO_3$), a commonly used food additive, and a compound that is generally regarded as safe (GRAS) by the United States Food and Drug Administration. Immersing fruit in solutions of SBC to control the postharvest incidence of *Penicillium digitatum* on citrus was first described in 1928 (Barger, 1928) and has since been used to control postharvest decay of lemons in California because it is inexpensive, readily available and can be used with little risk of fruit injury (Palou et al., 2001).

Many alternatives to chemical control have been investigated, but none, when used alone, provides the level of control of synthetic fungicides. While heat treatment virtually eliminates decay if fruit are inoculated prior to heating, it has little effect when infection occurs after heating, therefore, having no protective effect (Klein et al., 1997). Likewise, SBC does not provide persistent protection of the fruit from re-infection after treatment (Smilanick et al., 1999). The major limitations with biocontrol are a lack of eradicative activity, and a narrower spectrum of activity than is found with synthetic fungicides. The effect of environmental factors on biological control (performance margin) is also generally greater than for fungicides. As fruit mature, higher concentrations of the biocontrol antagonist must be used to achieve the same level of control as on immature fruit (Janisiewicz, unpublished data).

A combination of the three methods described above may complement one another to overcome the shortcomings of each. An antagonist, *Metchnikowia pulcherrima* T5-A2, was used in combination with heat (38 °C for 4 days) and 1-methylcyclopropene treatments to control decay caused by *P. expansum* and *C. acutatum* on 'Golden Delicious' apples under controlled atmosphere conditions (Janisiewicz et al., 2003). Both of these decays were effectively controlled by a combination of the antagonist and heat treatment. The antagonist controlled decay caused by *C. acutatum* more effectively than that caused by *P. expansum*, while *P. expansum* was more effectively controlled by heat treatment. In another study, *Bacillus subtilis* isolates were evaluated for control of postharvest decay of oranges by *P. digitatum* and *Penicillium italicum* and an increase in biocontrol activity of all isolates was observed when combined with SBC (Obagwu and Korsten, 2002). A combination of an antagonist and SBC also controlled *P. digitatum* on oranges and grapefruit more effectively than the either treatment alone (Porat et al., 2003).

The ultimate goal of this research is to devise a strategy that combines several of these alternatives that will equal the effectiveness of chemical control. The specific objective of this research was to determine the

effect of heat, antagonist, and SBC treatments, alone and in combination, on postharvest decay caused by *C. acutatum* and *P. expansum* on 'Golden Delicious' apples.

2. Materials and methods

2.1. Fruit

'Golden Delicious' apples were harvested at the preclimacteric stage (the climacteric rise in CO_2 and ethylene production had not yet begun) from a commercial orchard in Pennsylvania and randomized prior to treatment. The respiration and ethylene production rates of the preclimacteric fruit were 70 ± 5 nmol kg^{-1} s^{-1} and 3 ± 1 pmol kg^{-1} s^{-1}, respectively.

2.1.1. Fruit quality determination

Ethylene production and respiration rates of the non-heated control and heat-treated fruit were monitored every 8 h during a seven-day period using an automated system (Izumi et al., 1996). Four 5-fruit replications were measured after zero, two and four months in air storage at 0 °C. The starch content at harvest before and after heat treatment was measured using the Cornell generic starch scale of 1–8 (Blanpied and Silsby, 1992).

Magness–Taylor (MT) firmness measurements were done on 20 apples after zero, two and four months at 0 °C plus 1 day at 20 °C in air storage. Firmness was measured using a manually controlled digital penetrometer (EPT-1 with an 11.1-mm diameter tip; Lake City Technical Products, Kelowna, BC, Canada) set in the MT mode and interfaced with a personal computer. Magness–Taylor firmness (bioforce yield) was measured at two opposite points on the equator of each fruit after removing a thin slice of peel from each site.

2.2. Pathogens

The *C. acutatum* isolate was obtained from Kenneth D. Hickey, Penn State Fruit Research Laboratory and Extension Center, Biglerville, Pennsylvania, and had been used in a previous biocontrol test (Janisiewicz et al., 2003). The *P. expansum* isolate (MD-8) is a very aggressive isolate from our collection that was isolated from a decayed apple in storage and had also been used in previous biocontrol studies (Janisiewicz et al., 2003; Janisiewicz and Jeffers, 1997; Janisiewicz and Marchi, 1992). The pathogens were routinely grown on potato–dextrose–agar (PDA) and virulence was maintained by periodic transfers through apple. The conidial suspensions (1×10^4 conidia/ml) used to inoculate the fruit were prepared from 10-day-old cultures as previously described (Janisiewicz and Marchi, 1992).

2.3. Antagonists

The two antagonists (ST1-D9 and FMB-24H-2) used, were different strains of the yeast *M. pulcherrima* and were isolated from apple surfaces. Both strains were able to grow at low temperatures and were effective antagonists of *P. expansum*, (Janisiewicz et al., 2001) but had not been tested against *C. acutatum*. ST1-D9 was heat resistant at 38 °C (Leverentz et al., 2000), but FMB-24H-2 had not been tested for heat resistance. The yeasts were grown in 50 ml of nutrient yeast–dextrose broth medium in 250-ml Erlenmeyer flasks at 26 °C on a gyratory shaker at 150 rpm. After 24 h of incubation, the cells were harvested by centrifuging at $7000 \times g$ for 10 min, resuspending in water, and the concentration was adjusted to 3×10^7 CFU ml^{-1} with a spectrophotometer set at 420 nm.

2.4. Sodium bicarbonate

Solutions of SBC (Sigma–Aldrich, St. Louis, MO) at concentrations of 0 (control, water only), 0.3, or 1% (w/v) at pH 8.3–8.6 were used. The concentrations were selected following preliminary experiments to determine their compatibility with the two antagonists.

2.5. Fruit inoculation and treatments

2.5.1. Fruit inoculation and lesion measurement

The fruit were wounded with a six-penny nail to a depth of 4 mm and the wounds were inoculated with 25 μl per wound of either *C. acutatum* or *P. expansum* conidia alone, or the individual pathogens in combination with one of the antagonists and/or one of the SBC solutions. After inoculation, one lot of fruit was placed in 0 °C storage and another similarly treated

lot was subjected to heat treatment. Following heat treatment, the fruit were also stored at 0 °C in a completely randomized design and both lots of fruit were evaluated for decay incidence and severity after two and four months at 0 °C and again after an additional 14 days at 20 °C. The non-treated (control) fruit that were totally decayed by *P. expansum* after two months in storage were discarded after evaluation. There were three replications of 15 fruit per treatment. Decay severity was determined by measuring the diameter of the lesions at each evaluation period.

2.5.2. Heat treatment

The lot of apples to be heat treated was placed in tray-packed boxes containing perforated polyethylene bags as liners and heated in a thermostatically controlled (±1 °C) walk-in chamber. The fruit were heat treated at 38 °C for 4 days and the relative humidity was maintained at >85%. The storage conditions were monitored with a hygrothermograph (Belfort Instrument Co., Baltimore, MD). Following heat treatment, the fruit were stored at 0 °C.

2.6. Antagonist recovery

Populations of the two yeasts were determined immediately following inoculation (T_0), after 12 h at 20 °C following inoculation, and after the four-day heat treatment (38 °C), or cold storage (0 °C). Samples were also taken after two months and again after four months of storage in air at 0 °C and then after two additional weeks at 20 °C following four months cold storage. Pathogen populations were sampled from the wounds of four fruit per treatment, according to a procedure previously described (Leverentz et al., 2000).

2.7. Statistical analysis

2.7.1. Lesion severity and incidence

Decay severity due to either pathogen was determined by measuring the diameter of the lesion at each evaluation. No significant decay developed on the fruit inoculated with *C. acutatum* after four months at 0 °C, so the fruit were stored at 20 °C for an additional two weeks before determining decay incidence and severity (Fig. 1). The data was analyzed as a three-factor linear model using PROC MIXED (SAS Inst.) (Table 2). The assumptions of the mixed linear model were checked, and the variance grouping technique was used to correct for variance heterogeneity. The mean comparisons were done with Sidak adjusted *P*-values so that the experiment-wise error rate was 0.05. For the lesion incidence, a χ^2-analysis of the treatments was done using STATXACT 5 (Cytel Software Corp., 2000).

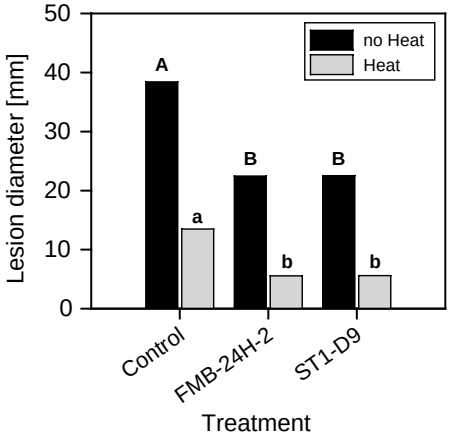


Fig. 1. Decay severity on 'Golden Delicious' apples inoculated with *Colletotrichum acutatum* and then subjected to various treatments or treatment combinations of heat (38 °C, 4 days), one of two biocontrol strains (FMB-24H-2 or ST1-D9) of *Metchnikowia pulcherrima* or sodium bicarbonate and then stored for 4 months at 0 °C plus 2 weeks at 20 °C. The means are averaged over all of the sodium bicarbonate treatments, since there was no sodium bicarbonate effect. Bars with different letters are statistically different at the significance level of 0.05. Lower case letters indicate statistical differences for the heat-treated fruit and capital letters indicate statistical differences for the non-heated fruit.

2.8. Antagonist recovery

The recovery data from apple wounds treated with FMB-24H-2 and ST1-D9 were analyzed as a three factor linear model (where applicable) using PROC MIXED (SAS Inst.) with time, antagonist and SBC as the main effects. The assumptions of the mixed linear model were checked and the variance grouping technique was used to correct variance heterogeneity. The mean comparisons were done with Sidak adjusted *P*-values so that the experiment-wise error rate was 0.05. The data were analyzed separately for each storage time.

3. Results

3.1. Fruit physiology

Between 2 and 3 days after harvest, the fruit entered the climacteric stage of development, as indicated by rapid increases in the respiration and ethylene production rates (data not shown). Heat treatment reduced climacteric respiration and ethylene production rates following zero and two months at 0 °C plus 7 days at 20 °C and four months at 0 °C plus 1 or 14 days at 20 °C (Table 1). While heat treatment initially decreased fruit firmness, heat-treated fruit were firmer than non-heated fruit following zero and two months at 0 °C plus 7 days at 20 °C and four months at 0 °C plus 1 or 14 days at 20 °C. Heat treatment increased the starch score, i.e., decreased the starch content of the fruit at harvest (Table 1).

3.2. Effect of treatments on decay

3.2.1. Decay severity

Since little decay developed on fruit inoculated with *C. acutatum* after two or four months at 0 °C, the fruit inoculated with this pathogen were moved to 20 °C for two weeks during which time significant decay developed. Heat treatment had a significantly greater effect in reducing decay than either antagonist (Fig. 1). The mean lesion diameters on the non-heated or heat-treated fruit were 38.41 mm and 13.49 mm, respectively. The lesion diameters of the

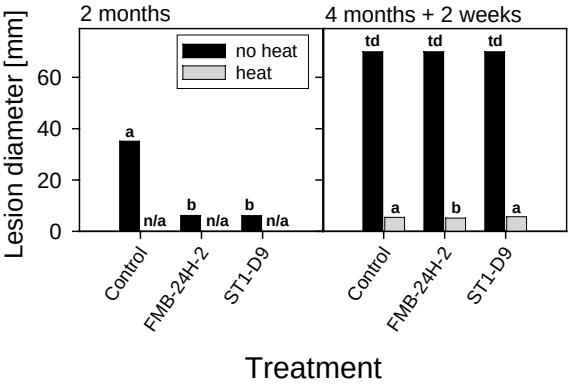


Fig. 2. Decay severity on 'Golden Delicious' apples inoculated with *Penicillium expansum* and then subjected to various treatments or treatment combinations of heat (38 °C, 4 days), one of two biocontrol strains (FMB-24H-2 or STI-D9) of *Metchnikowia pulcherrima* or sodium bicarbonate after 2 months at 0 °C and after 4 months at 0 °C plus 2 weeks at 20 °C. The means are averaged over all of the sodium bicarbonate treatments, since there was no sodium bicarbonate effect. Values for fruit that had either total decay (td) or no lesions (n/a = not analyzed) were not included in the statistical analysis. Bars with different letters within storage time are significantly different ($P = 0.05$).

FMB-24H-2 and ST1-D9 treated fruit were 22.45 mm and 22.53 mm, respectively, with both antagonists being equally effective in reducing decay. Heat in combination with either antagonist provided significantly better control than either heat or the antagonists alone. The mean lesion diameters of the heat × FMB-24H-2 and heat × ST1-D9 were 5.55 mm and

Table 1
Maturity indices of non-heated and heat-treated 'Golden Delicious' apples stored in air at 0 °C for various periods of time

Treatment	CO_2 production (nmol kg^{-1} s^{-1})	Ethylene production (nmol kg^{-1} s^{-1})	Firmness (N)	Starch
At harvest (after onset of the climacteric)				
Non-heated	173.6 ± 6.9 a[a]	1.36 ± 0.09 a	97.0 ± 8.0 a	3.8 ± 0.9 a
Heated	151.7 ± 4.0 b	1.27 ± 0.03 b	86.8 ± 7.5 b	6.2 ± 1.0 b
2-month-cold storage plus 7 days at 20 °C				
Non-heated	153.2 ± 13.0 a	1.32 ± 0.05 a	68.3 ± 12.4 b	
Heated	129.5 ± 7.0 b	1.00 ± 0.07 b	75.3 ± 9.1 a	
4-month-cold storage plus 1 day at 20 °C				
Non-heated	175.2 ± 3.8 a	1.22 ± 0.16 a	64.5 ± 6.1 b	
Heated	140.0 ± 13.4 b	0.75 ± 0.06 b	70.3 ± 7.0 a	
4-month-cold storage plus 14 days at 20 °C				
Non-heated	141.9 ± 5.4 a	1.31 ± 0.14 a	59.2 ± 5.3 b	
Heated	116.5 ± 12.8 b	1.04 ± 0.04 b	65.7 ± 6.0 a	

[a] Within columns for each time period, values labeled with the same letter are not significantly different at $\alpha = 0.05$ using Tukey's HSD.

5.59 mm, respectively. There was very little effect of SBC alone or interacting with heat or antagonists alone or in combination.

P. expansum caused decay at all rating times (Fig. 2). Heat treatment was the most effective treatment and no decay developed on heat-treated fruit after two months at 0 °C, thus the heat-treated fruit were not included in the two month statistical analysis. On non-heated fruit, both antagonists reduced decay significantly, but there was no difference in effectiveness between the two antagonists. The mean lesion diameter of the control was 35.11 mm, while those of the antagonists, FMB-24H-2 and ST1-D9, were 6.18 and 6.11 mm, respectively. After four months of cold storage, all of the control fruit were totally decayed, and therefore discarded. There was no significant effect of the SBC treatments in combination with the antagonists. On non-heated apples the SBC × heat effects were significant ($F = 6.39$, $P = 0.0020$). However, there was no difference in decay between the two concentrations of SBC (Fig. 3). On heated apples there was no SBC × antagonist effect, although there was a trend toward less decay on fruit when SBC was combined with the antagonist, but the differences were too small to be statistically significant. Only the heated fruit were retained after four months at 0 °C plus two weeks at 20 °C (Fig. 2). At that time, the antagonist effect was significant with FMB-24H-2 being more effective in reducing decay than ST1-D9, but the difference here was also very small, because the lesions were not very large.

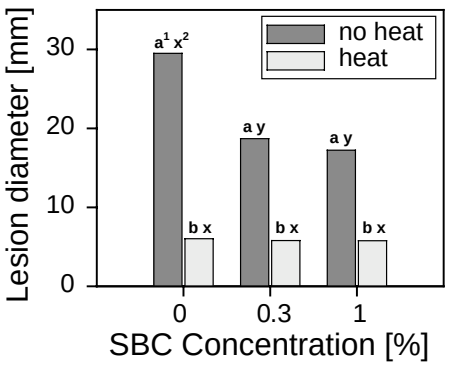


Fig. 3. Decay severity on 'Golden Delicious' apples inoculated with *Penicillium expansum* and then treated with sodium bicarbonate (SBC) at 0, 0.3, or 1.0% and either of the biocontrol strains (FMB-24H-2 or ST1-D) of *Metchnikowia pulcherrima* and then heated (38 °C, 4 d) or transferred directly to cold storage in air at 0 °C. Fruit had been stored for 4 months at 0 °C. [1]Treatment means within SBC concentrations with different letters (a, b) are different at the 0.05 significance level; [2]Treatment means within heat or no heat treatments with different letters (x, y) are different at the 0.05 significance level. Values for fruit that had total decay (td) were not included in the statistical analysis. The means are averaged over both antagonists since the effect of either antagonist was similar.

3.2.2. Decay incidence

Decay incidence was rated by determining the number of fruit inoculated with *C. acutatum* or *P. expansum* that had no lesions. The number of *C. acutatum*-inoculated apples without lesions by treatment and storage time is shown in Table 2. A chi-square analysis of the 18 treatments using STATXACT 5 (Cytel Software Corp.) showed that the frequency distributions were not all the same (Chi-square = 256.7, *P*-value = 0.0005). The top six or best treatments were not statistically different (Chi-square = 8.0000, *P*-value = 0.1571). However, the top seven treatments were significantly different

Table 2
Number of apples without decay (out of 45 per treatment) at various sampling times on fruit inoculated with *C. acutatum* and subjected to various treatments

Treatment			Sampling time		
Antagonist[a]	SBC	Heat	2 Months	4 Months	4 Months + 2 weeks
S	0.3[b]	+	45	42	37
S	0.0	+	43	38	36
F	0.3	+	45	41	35
F	1.0	+	45	39	34
S	1.0	+	45	38	30
F	0.0	+	45	40	26
C	0.3	+	45	40	18
C	0.0	+	45	40	14
C	1.0	+	44	41	9
F	0.3	−	45	45	4
F	1.0	−	45	44	3
S	0.0	−	45	43	3
S	1.0	−	45	44	2
S	0.3	−	45	43	1
F	0.0	−	44	42	1
C	0.3	−	45	40	0
C	0.0	−	45	39	0
C	1.0	−	38	31	0

[a] S, antagonist ST1-D9; F, antagonist FMB-24H-2; C, control.
[b] Concentration (%) of sodium bicarbonate (SBC).

Table 3
Number of apples without decay (out of 45 per treatment) at various sampling times on fruit inoculated with *P. expansum* and subjected to various treatments

Treatment			Sampling time		
Antagonist[a]	SBC	Heat	2 Months	4 Months	4 Months + 2 weeks
S	1.0[b]	+	45	39	39
S	0.3	+	43	39	37
F	0.0	+	45	41	34
F	1.0	+	45	39	33
F	0.3	+	45	43	32
S	0.0	+	43	34	31
C	0.3	+	45	36	17
C	0.0	+	45	35	15
C	1.0	+	44	35	15
F	1.0	–	39	25	8
S	0.3	–	38	20	7
F	0.3	–	43	29	6
S	0.0	–	38	16	6
S	1.0	–	42	25	5
F	0.0	–	35	16	4
C	0.0	–	0	0	0
C	0.3	–	0	0	0
C	1.0	–	0	0	0

[a] S, antagonist ST1-D9; F, antagonist FMB-24H-2; C, control.
[b] Concentration (%) of sodium bicarbonate (SBC).

(Chi-square = 21.94, *P*-value = 0.0013). The antagonists plus heat were the most effective treatments and were not different from each other. The heat treatment alone was more effective than the antagonists alone. On non-heated fruit, both antagonists significantly reduced decay. SBC had no effect on the incidence of decay.

For *P. expansum* (Table 3), a Chi-square analysis of the 15 non-zero treatments showed that the frequency distributions were not all the same (Chi-square = 215.4, *P*-value = 0.0005). Further analysis showed that the top six treatments were not statistically different (Chi-square = 5.002, *P*-value = 0.4156). However, the top seven treatments were statistically different (Chi-square = 26.43, *P*-value = 0.0003). As with *C. acutatum*, all of the antagonists plus heat were the most effective treatments and were not different from each other. The heat treatment alone was more effective than the antagonists alone, but on non-heated fruit, the apples treated with the antagonists had significantly less decay than the controls. Here too, SBC had no effect on decay incidence.

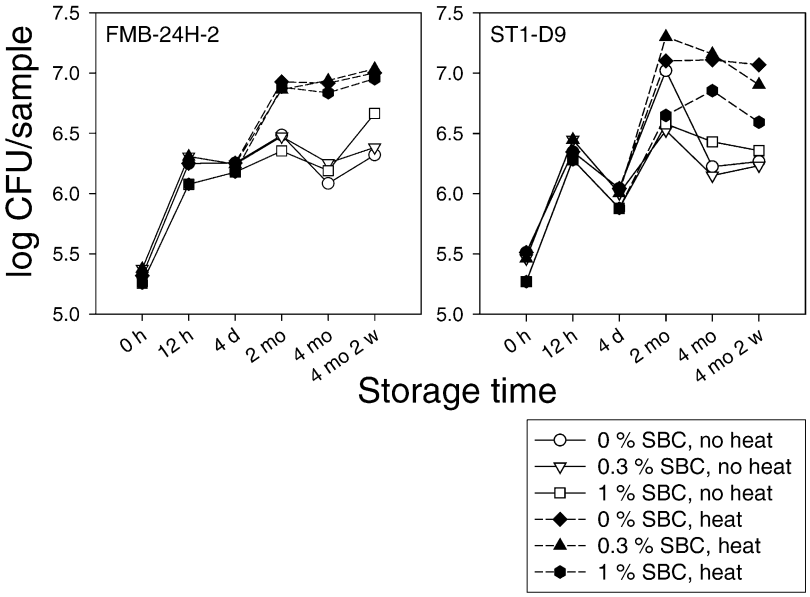
3.3. Antagonist recovery

The populations of the antagonists increased over one log unit during the first 12 h and then were stable throughout the study (Fig. 4). The heat resistance of FMB-24H-2 was similar to that of ST1-D9. Following two months cold storage and thereafter, the populations of both antagonists on the heat-treated fruit were higher than on non-heated fruit.

4. Discussion

'Golden Delicious' apple fruit inoculated with *C. acutatum* did not decay during storage at 0 °C for four months, confirming earlier observations with this pathogen (Janisiewicz et al., 2003). Therefore, fruit were stored at 20 °C for an additional two weeks to allow decay to develop so that the effectiveness of the various treatments could be determined. *P. expansum*, however, caused extensive decay, even under cold storage conditions, indicating that it is a much more aggressive pathogen than *C. acutatum*. No growth of *C. acutatum* on PDA plates was observed at 0 °C, but growth resumed when the plates were subsequently moved to 20 °C (data not shown). Thus, the low temperature was probably the main factor in preventing decay development caused by this fungus during the four months of cold storage. *M. pulcherrima* strains FMB-24H-2, ST1-D9, and T5-A2, among others, have all been shown to be effective in reducing decay caused by *P. expansum* (Janisiewicz et al., 2001; Janisiewicz et al., 2003). In a recent study combining an antagonist and heat treatment (Janisiewicz et al., 2003), strain T5-A2 alone effectively reduced decay caused by *C. acutatum* and *P. expansum* and was even more effective in combination with heat. Similarly, in the present study, strains FMB-24H-2 and ST1-D9 reduced decay caused by *C. acutatum* and the decay was further reduced in combination with heat. Heat alone was very effective in reducing decay severity caused by *P. expansum*, but combining heat with either of the two antagonists was necessary in order to have a similar effect on *C. acutatum*. In this study, FMB-24H-2 had similar heat resistance to that of ST1-D9, which had previously been tested (Leverentz et al., 2000).

The mode of action of the heat treatment seems to be both through direct interaction with the fungus



	0 h	12 h	4 d	2 months	4 months	4 months + 2 weeks
no heat	--	--	--	6.5 a	6.2 a	6.3 a
heat	--	--	--	6.9 b	6.9 b	6.9 b

Fig. 4. Recovery of *Metchnikowia pulcherrima* strains FMB-24H-2 or ST1-D9 from 'Golden Delicious' apples which were heated (38 °C, 4 days) or non-heated and/or treated with 0, 0.3, or 1.0% solutions of sodium bicarbonate (SBC) and stored for various times (0 h = 0 hours; 12 h = 12 hours at 20 °C; 4 d = 4 days in heat or at 20 °C; 2 mo = 2 months at 0 °C; 4 mo = 4 months at 0 °C; 4 mo 2w = 4 months at 0 °C plus 2 weeks at 20 °C). The statistical analysis (table) combines the means of the two antagonists within each sampling time.

itself, and via physiological responses of the fruit tissue. In vitro studies showed that both germination and growth declined when fungi were exposed to extended periods at higher temperatures. Heat treatment may also alter the susceptibility of the host to pathogens by the formation of an inhibitory substance in the peel (Fallik et al., 1995). In addition to its ability to reduce decay, heat treatment has beneficial effects on modifying ripening. While seemingly hastening ripening, as indicated by enhanced peel degreening (Klein and Lurie, 1992; Liu, 1978), postharvest heat treatment actually delays other important ripening characteristics involved with maintaining fruit quality in storage by transiently inhibiting volatile production (Fallik et al., 1997) and maintaining fruit firmness (Klein and Lurie, 1992; Liu, 1978).

Recent research on the effect of SBC in reducing postharvest fruit decay has mainly focused on citrus fruit (Obagwu and Korsten, 2002; Palou et al., 2001; Porat et al., 2003; Smilanick et al., 1999). Significant control of *P. italicum* was achieved by 2, 3, and 4% solutions of SBC at room temperature, while 1% was ineffective (Palou et al., 2001). The SBC treatment was thought to be primarily fungistatic in that it did not kill the *P. italicum* spores, and therefore was not very persistent since the fungus survived the treatment. The fungistatic effect was thought to be due to the presence of bicarbonate residues in the wounds. The presence of SBC delayed the germination of spores in the treated wounds. A more recent study showed that a 2% SBC solution killed germinating *P. digitatum* spores in wounds of citrus fruits (Porat et al., 2003). Apparently, germinating spores are more readily killed by SBC than non-germinating spores (Marloth, 1931). Also, combining an SBC-tolerant strain of the antagonist *B. subtilis* with SBC improved decay control of *P. digitatum* and *P. italicum* (Obagwu and Korsten, 2002). It was concluded that the space created by the disruption of the pathogen development at the wound site by SBC may have given the antagonist a competitive

advantage. This may also have been the case in this current study on non-heated, SBC treated fruit, while the heat treatment may have disrupted the pathogen enough to give the antagonists a competitive advantage on heated fruit.

Smilanick et al. (1999) significantly improved the control of *P. digitatum* on citrus fruits by combining *Pseudomonas syringae* strain ESC10 (the active ingredient in BioSave 10; Village Farms, BioSave Division, Orlando, FL) with a 3% SBC solution. The 0.3 and 1% SBC solutions used in the present study were chosen to minimize the effect of SBC on FMB-24H-2 or ST1-D9. The studies cited above, all used SBC solutions at concentrations of 2% or more, which were compatible with the antagonists used. The 0.3 and 1% SBC solutions in our study, though compatible with the antagonists used, may not have been of sufficient strength to negatively affect *C. acutatum*, but reduced decay caused by *P. expansum* after four months at 0 °C. The negative effect of SBC on *P. expansum* was detected only on non-heated fruit. If heat was used, treatment with SBC was unnecessary.

The need for finding suitable alternatives to fungicides to control postharvest decay has prompted research aimed at combining various alternatives into a control strategy that equals the effectiveness of synthetic chemicals. The ideal strategy would eradicate any pathogens present at the time of treatment and protect the commodity from further infection. Most of the alternatives currently being investigated do not have both eradicant and protectant capabilities. Our study shows that combining several alternatives may result in a successful control strategy that includes the desired capabilities, and in addition, we have shown that FMB-24H-2 can be added to the list of heat-resistant biocontrol agents which can effectively reduce decay caused by postharvest pathogens tested.

Acknowledgements

We would like to acknowledge Willard Douglas and Corrine Walters for valuable technical assistance.

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